British Journal of Anaesthesia 1997; 79: 567–574

Pharmacokinetic–pharmacodynamic modelling of the EEG effects of Ro 48-6791, a new short-acting benzodiazepine, in young and elderly subjects

J. DINGEMANSE, J. HÄUSSLER, W. HERING, H. IHMSEN, S. ALBRECHT, M. ZELL, H. SCHWILDEN AND J. SCHÜTTLER

Summary

The objectives of this study were to explore, by a modelling approach, in nine young (24–28 yr) and nine elderly (67–81 yr) male subjects, the pharmacokinetics and pharmacodynamics of Ro 48-6791, a new water soluble benzodiazepine. A microprocessor-controlled i.v. infusion pump generated linearly increasing arterial plasma concentrations until predetermined EEG and clinical end-points were attained. This concentration was maintained for 15 min and thereafter the infusion was discontinued. Haemodynamic and respiratory variables were monitored continuously. At full reorientation of the subject, a second infusion cycle was started under the same conditions to investigate the reproducibility of the concentration–effect relationship. The plasma concentration–time profiles of Ro 48-6791 were fitted accurately to an open three-compartment model. Plasma concentrations of Ro 48-6792, an N-dealkylated metabolite, accumulated during the course of the study. Pharmacokinetic variables of Ro 48-6791 were similar for both groups. The largest differences between young and elderly subjects, respectively, were found for clearance (mean 85 (SD 23) *vs* 71 (15) litre h^{-1}) and k_{12} (11 (7) *vs* 7 (3) h^{-1}). The concentration–median EEG frequency relationship was described with a sigmoid Emax model. Elderly subjects showed slightly increased drug sensitivity compared with young subjects (EC_{50} 72 (25) and 44 (15) μg $litre^{-1}$ in young and elderly subjects, respectively). The concentration–response data of the second infusion cycle deviated from the fitted curve suggesting either development of acute tolerance to the EEG effects of Ro 48-6791 or a role for drug metabolites. Because of the differences in sensitivity and clearance, lower doses of Ro 48-6791 should be administered to elderly compared with young subjects in order to achieve similar effects. (*Br. J. Anaesth.* 1997; **79**: 567–574).

Key words

Hypnotics benzodiazepine, Ro 48-6791. Pharmacokinetics, Ro 48-6791. Pharmacodynamics, Ro 48-6791. Age factors. Monitoring, electroencephalography.

Benzodiazepines are in widespread use as sedative–hypnotic agents.[1] Parenteral midazolam and other benzodiazepines are currently the most frequently used sedatives for conscious sedation.[2] Midazolam is also in widespread use for both induction and maintenance of general anaesthesia[3] and it is frequently administered for long-term sedation of critically ill patients in intensive care units.[4]

Midazolam is considered by many practitioners to be associated with a longer duration of action and greater inter-individual variability of effect than is desirable.[5,6] A sedative drug with the advantages of midazolam but with a shorter duration of action and less variability would be of great benefit.

Ro 48-6791 (3-(5-dipropylaminomethyl-1,2,4-oxadiazol-3-yl)-8-fluoro-5-methyl-5,6-dihydro-4H-imidazo [1,5-a][1,4] benzodiazepin-6-one) (fig. 1) is a new water soluble full agonist at the benzodiazepine receptor. In the first study in humans, in which doses of 0.1–3 mg were given i.v. over 20 min, Ro 48-6791 was shown to be well tolerated and to elicit dose-dependent central nervous system depressant effects.[7] An integrated pharmacokinetic–pharmacodynamic approach[8] applying neurophysiological effect measures (saccadic eye movements and electroencephalography β-power) provided pivotal data for further clinical development of Ro 48-6791. The compound had a comparable onset and duration of action to midazolam but was approximately five-fold more potent[7] and its volume of distribution and plasma clearance were larger than those of midazolam. The monopropyl metabolite Ro 48-6792 (fig. 1) rapidly appeared in plasma and seemed to have a longer elimination half-life than the parent compound.

The objectives of this study were to investigate in

JASPER DINGEMANSE*, PHD, Department of Clinical Research, F. Hoffmann-La Roche, Basel, Switzerland and Jacor Research, Bottmingen, Switzerland. JÜRGEN HÄUSSLER, MD, MANFRED ZELL, MSC, Department of Clinical Research, F. Hoffmann-La Roche, Basel, Switzerland. WERNER HERING, MD, HARALD IHMSEN, MSC, SVEN ALBRECHT, MD, JÜRGEN SCHÜTTLER, MD, Department of Anaesthesiology, University of Erlangen, Erlangen, Germany. HELMUT SCHWILDEN, MD, PHD, Department of Anaesthesiology, University of Bonn, Bonn, Germany.
Accepted for publication: July 14, 1997.
*Address for correspondence: Jacor Research, Buchenstrasse 2, 4103 Bottmingen, Switzerland.

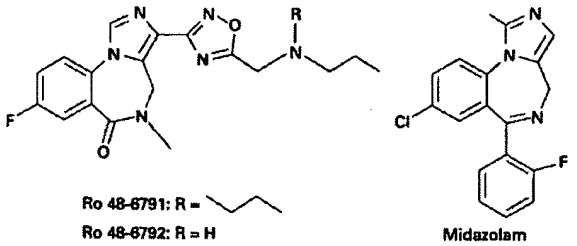

Figure 1 Chemical structures of Ro 48-6791 and its N-dealkylated metabolite Ro 48-6792. The structure of midazolam is given for comparison.

both young and elderly subjects the pharmacokinetics and pharmacodynamics of Ro 48-6791 in a concentration range for potential use in anaesthesia rather than conscious sedation. Arterial blood samples were collected during and after linearly ascending blood concentrations to exploit the advantages of pharmacokinetic–pharmacodynamic modelling approaches.[9,10] After recovery from the effects of the first infusion cycle, the drug was again infused until the same predetermined pharmacodynamic end-point was reached. This strategy was designed to evaluate the potential contribution of the metabolite Ro 48-6792 to the overall drug effects.

Subjects and methods

We studied 18 male subjects, nine young (age 24–28 yr, weight 66–89 kg) and nine elderly (age 67–81 yr, weight 71–90 kg), regarded as healthy on the basis of medical history, physical–neurological examination, 12-lead ECG and clinical laboratory measurements. Subjects were non-smokers and absence of drug abuse (including benzodiazepines) was verified by urinary screening. Three elderly subjects were receiving antihypertensive drugs and one was also receiving treatment for chronic heart failure. All subjects gave written informed consent before any screening procedures were performed. The study was approved by the Medical Ethics Review Committee of the Medical Faculty of the University of Erlangen, Erlangen, Germany.

STUDY DESIGN

All 18 subjects received treatment with Ro 48-6791. For safety reasons, the young subjects were treated before the elderly subjects. Commonly, two subjects were treated on one day. Subjects fasted for at least 8 h before the start of administration of the study drug until full orientation after the second infusion cycle. To avoid hypoglycaemia, Ringer's solution with glucose was infused during the study. The drug was given i.v. with a microprocessor-controlled (Toshiba 1850 C) infusion pump (Braun Perfusor fm, Braun, Melsungen, Germany) yielding linearly increasing plasma concentrations. The infusion rate was programmed to generate an increase in plasma concentration of 180 and 90 $\mu g\ litre^{-1}\ h^{-1}$ in the young and elderly subjects, respectively. The slope of the plasma concentration increase in the elderly was reduced for safety reasons and anticipated increased drug sensitivity.[11,12] Pharmacokinetic variables obtained in the entry-into-human study[7] were used to program the software (IVA-PUMP, version 3.5., Erlangen, Germany). The study drug was infused until the median electroencephalographic (EEG) frequency was less than 4 Hz and the subject ceased to respond to a standardized loud acoustic stimulus. The concentration at which the sedation criteria were fulfilled was maintained for another 15 min before the infusion was terminated. As soon as the subject was fully oriented with regard to person, place and time, a second infusion cycle was started under the same conditions and with the same assessments.

PROCEDURES AND ASSESSMENTS

After ulnar artery blood supply had been examined by the Allen test, a cannula (Arrow, 20-G) was inserted by the Seldinger technique under local anaesthesia (Scandicain 1%, Astra Chemicals) into the radial artery of the non-dominant arm. Another cannula (Viggo, 18-G) was inserted into a forearm vein of the contralateral side for infusion of drug and glucose solution. Haemodynamic and EEG monitoring devices were installed and after a rest of approximately 10 min, stable baseline values of arterial pressure, heart rate, oxygen saturation and EEG were recorded.

Safety and tolerability

Adverse events were assessed by spontaneous reports, observations and questioning at regular intervals. The intensity of the adverse event was rated on a three-point scale (mild, moderate, severe). Systolic, diastolic and mean arterial pressures, and heart rate were monitored continuously via an arterial cannula (Sirecust 1281, Siemens) and the ECG was monitored continuously (Sirecust 1281, Siemens). Oral body temperature was measured just before start of the first infusion cycle, once during each period of constant plasma concentration and at the time of full orientation after the second cycle. Respiratory function was monitored continuously by pulse oximetry. When oxygen saturation decreased to less than 93% and 87% in young and elderly subjects, respectively, oxygen was administered by mask at a flow rate of 3–4 litre min^{-1}. During the phases of constant plasma concentration, an arterial blood sample was analysed for blood-gas tensions (BMS SMK2, Radiometer Copenhagen). After termination of the study, a physical examination and routine clinical laboratory tests were performed again.

Analytics and pharmacokinetics

Blood samples of 2.5 ml were collected into Monovettes containing EDTA as anticoagulant via the indwelling arterial cannula. The dead space of the cannula (approximately 300 µl) was flushed with a small amount of physiological saline after each blood sample. Before obtaining each sample for analysis, 1 ml of blood was obtained and discarded. The two infusion cycles were divided into two

periods, periods A and B, and periods C and D, respectively. Period A was the first infusion period (including the plateau phase) followed by period B, the first recovery period, terminated by complete reorientation of the volunteer. Periods C and D were the respective phases of the second infusion cycle. During periods A and C, blood samples were obtained every 3–5 min. During periods B and D, blood was collected at increasing intervals of 2–30 and 2–60 min, respectively. In general, blood samples were collected for up to 6 h after termination of the second infusion with a total blood loss of approximately 160 ml per subject. After centrifugation, plasma samples were stored at −20 °C until analysis.

After precipitation of plasma proteins with acetonitrile, Ro 48-6791 and its metabolite Ro 48-6792 were extracted simultaneously from the supernatant on a trapping column using on-line solid phase extraction and separated on an analytical column with column-switching liquid chromatography. The effluent from the analytical column was passed directly to an ion spray interface coupled to a tandem mass spectrometer (LC-MS/MS). Mass spectrometric detection of the analytes was performed using selected reaction monitoring. Mean precision (coefficient of variation) and accuracy (percentage of real concentration present) for Ro 48-6791 and Ro 48-6792 in the concentration range 1.0–50 μg litre^{-1} were 6.1% and 101.7%, and 6.9% and 99.7%, respectively. The lower limit of quantification for both Ro 48-6791 and Ro 48-6792 was 0.5 μg litre^{-1}, using a 0.1-ml aliquot of plasma. Pharmacokinetic variables were calculated using standard techniques[13] and fitted to an open three-compartment model using the program NONMEM.[14]

Pharmacodynamics

EEG recording began 10 min before administration of Ro 48-6791 and was continued until the early second recovery period when subjects had regained full orientation. The EEG was recorded using the CATEEM electrodiagnostic monitoring system (Computer Aided Topographical Electroencephalometry, Medisyst, Linden, Germany). Bipolar cerebral activity was acquired using an electro-cap (Electro-Cap, Electro-Cap International, Eaton, OH, USA) with the electrodes positioned according to the international 10–20 system and using Cz as reference. The gap between the cavities of the electrodes and the tissue was bridged by an electrolytic jelly. The signals were preamplified with a battery-powered amplifier close to the head and the resulting digital code was transmitted by optical fibre to the CATEEM system. Artefact-free serial epochs of 4 s were digitized at a rate of 512 Hz (12 bit) using bandpass filters between 0.45 and 35 Hz. The high input impedance ensured a sufficient signal-to-noise ratio with electrode impedances ranging from 1 to 100 kΩ. An additional display of the unprocessed raw EEG was used to check the variables derived. For each lead, EEG analysis included changes within individual band power and median frequency, defined as the frequency which divides the area below the power spectrum curve into two equal parts. The individual frequency bands were set as follows: delta 1.25–4.5 Hz, theta 4.75–6.75 Hz, alpha$_1$ 7.0–9.5 Hz, alpha$_2$ 9.75–12.5 Hz, beta$_1$ 12.75–18.5 Hz and beta$_2$ 18.75–35.0 Hz. To correlate the EEG changes with plasma concentrations, the values of the central lead of the dominant hemisphere (C_3) were used from the 17 channels derived. As the CATEEM system excludes the subdelta band (0.5–1.25 Hz) from the power spectral analysis, the raw EEG signal of this lead was transferred to a separate PC-based analysis tool. There, the power spectrum was analysed from 0.5 to 32 Hz and median frequency calculated subsequently (EEG-BASIC, version 2.4, Bonn, Germany), thus receiving more information during the pronounced hypnotic effect. Median frequency was used as a pharmacodynamic effect measure for modelling purposes.

Pharmacokinetic–pharmacodynamic modelling

Visual inspection of individual plasma concentration–median EEG frequency curves showed that the relationship could most adequately be described by a sigmoid Emax model[15]:

$$E = E_0 \frac{Emax \times C_P^{\gamma}}{EC_{50}^{\gamma} + C_P^{\gamma}}$$

where E = median frequency (Hz); E_0 = baseline median frequency (Hz); Emax = maximum possible effect on median frequency attributable to the drug (Hz); C_P = plasma concentration of Ro 48-6791 (μg litre^{-1}); EC_{50} = plasma concentration of Ro 48-6791 (μg litre^{-1}) that gives 50% of the maximum attainable inhibition in median frequency; and γ = Hill coefficient, a variable which describes the steepness of the concentration–response relationship (no dimension).

Pharmacokinetic–pharmacodynamic modelling procedures were performed by incorporating a hypothetical effect compartment. The rate of drug transfer out of the effect compartment was characterized by the rate constant k_{eq} (h^{-1}). The half-life of equilibration ($T_{1/2eq}$) was calculated by ln $2/k_{eq}$. The pharmacodynamic model variables were estimated for the first infusion cycle by non-linear regression analysis using NONMEM and the optimum-fit pharmacokinetic variables.

STATISTICAL ANALYSIS

Differences between young and elderly volunteers were tested for statistical significance by the unpaired Student's t test. $P<0.05$ was considered statistically significant.

Results

SAFETY AND TOLERABILITY

All 18 subjects completed the study according to the design, without any serious adverse events. Apart from central depressant activity, few adverse events were reported. One young subject reported a fever

lasting for approximately 15 h, 1 day after treatment with Ro 48-6791. Mild atrial arrhythmia and excessive dreaming, for 5 min during the second infusion cycle and during the night after drug administration, respectively, were reported by two elderly subjects. In a few subjects oxygen saturation decreased to less than the threshold set, but normalized immediately after administration of oxygen by mask. All participants breathed spontaneously during the entire observation period. Systolic, diastolic and mean arterial pressures, and heart rate, remained stable during the study in all subjects. Body temperature transiently decreased slightly to less than 35 °C in two elderly subjects. There was no pattern of abnormal laboratory values during the study which suggested a treatment effect, and the abnormalities observed were judged to be clinically irrelevant. The investigations performed at the post-study screen did not reveal clinically relevant differences from the baseline situation.

PHARMACOKINETICS

Table 1 presents the duration of each infusion cycle, time interval between the two cycles and dose of Ro 48-6791 administered in each cycle. Figure 2 shows the plasma concentration–time courses of Ro 48-6791 and Ro 48-6792 in a representative elderly subject. The fitted curves, on the basis of a three-compartment model for Ro 48-6791 and a two-compartment model for Ro 48-6792, are also given. In the ascending part of the two infusion cycles the concentration of the parent drug increased linearly with time. After the predetermined pharmacodynamic end-point had been reached, relatively constant concentrations of Ro 48-6791 were maintained for 15 min in most subjects. The metabolite Ro 48-6792 appeared rapidly in plasma and surpassed concentrations of the parent drug shortly after termination of the first infusion.

During the second infusion cycle, plasma concentrations of Ro 48-6792 accumulated further and by the end of the second infusion were much higher than those of Ro 48-6791. Thereafter, concentrations of Ro 48-6792 declined slowly in comparison with those of Ro 48-6791. In both groups, Ro 48-6791 showed a high plasma clearance, small initial volume of distribution and relatively short half-life (table 2).

Table 1 Dose and duration of infusion of Ro 48-6791 in the two infusion cycles (mean (SD))

	Young	Elderly
Period A–B		
Duration (min)	42 (3)	43 (5)
Dose (mg)	15.4 (2.6)	8.4 (1.8)
Period C–D		
Duration (min)	34 (3)	38 (3)
Dose (mg)	11.0 (1.7)	6.9 (1.2)
Interval between cycles (min)	45 (10)	48 (11)
Total dose (mg)	26.4 (3.2)	15.2 (1.9)

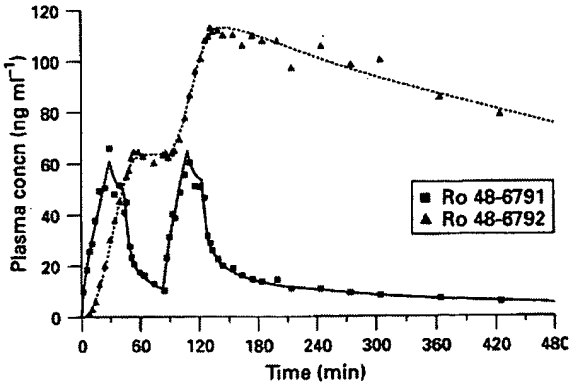

Figure 2 Actual and fitted plasma concentration–time course of Ro 48-6791 and Ro 48-6792 during and after both infusion cycles in a representative elderly subject (No. 101).

Table 2 Pharmacokinetic variables of Ro 48-6791 in young and elderly subjects analysed by an open three-compartment model (mean (SD))

	Young	Elderly
k_{12} (h^{-1})	11 (7)	7 (3)
k_{21} (h^{-1})	3.3 (1.3)	3.1 (0.9)
k_{13} (h^{-1})	3.3 (2.3)	3.8 (2.0)
k_{31} (h^{-1})	0.4 (0.3)	0.4 (0.1)
Cl_1 (litre h^{-1})	85 (23)	71 (15)
Cl_2 (litre h^{-1})	189 (47)	131 (33)
Cl_3 (litre h^{-1})	56 (22)	67 (29)
V_1 (litre)	20.5 (7.1)	19.5 (5.8)
V_2 (litre)	60.7 (14.8)	43.8 (13.0)
V_3 (litre)	152 (74)	162 (62)
V_{ss} (litre)	233 (80)	226 (57)
$T_{1/2}^{\alpha}$ (h)	0.041 (0.019)	0.046 (0.016)
$T_{1/2}^{\beta}$ (h)	0.56 (0.19)	0.49 (0.19)
$T_{1/2}^{\gamma}$ (h)	3.8 (1.4)	3.7 (0.8)

PHARMACODYNAMICS

The time course of the changes in median EEG frequency during and after infusion of Ro 48-6791 in an elderly subject are shown in figure 3. Baseline median EEG frequency was approximately 7–9 Hz in all subjects. After the start of the first infusion, approximately 12 subjects initially showed a slight increase in median frequency. Relatively shortly before the pharmacodynamic end-point was reached, the effect variable decreased sharply to values close to 2 Hz. The time course of the return to baseline values in both recovery periods was steep. It

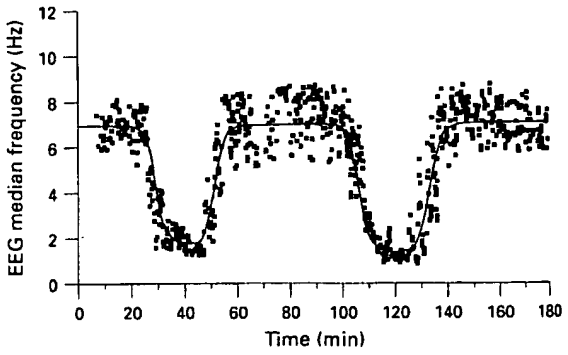

Figure 3 Median EEG frequency as a function of time during and after both infusion cycles in an elderly subject (No. 101).

Table 3 Pharmacodynamic variables of Ro 48-6791 in young and elderly subjects on the basis of a sigmoid Emax model and a hypothetical effect compartment. Values are means (SD) [median] of $n=9$. *$P<0.05$ compared with young subjects

	Young	Elderly
E_0 (Hz)	8.6 (2.4) [8.5]	7.4 (2.2) [7.0]
Emax (Hz)	7.0 (1.9) [6.9]	5.4 (1.3) [5.9]
EC_{50} (μg litre^{-1})	72 (25) [79]	44 (15) [42]*
γ	11 (7) [14]	34 (19) [45]
k_{eq} (h^{-1})	10 (6) [8]	14 (22) [6]

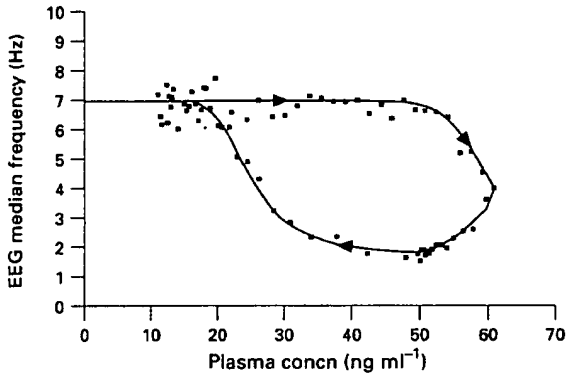

Figure 4 Relationship between arterial plasma concentration and median EEG frequency during the first infusion cycle in elderly subject No. 101.

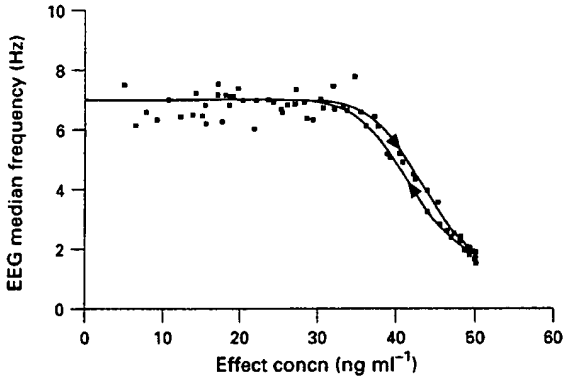

Figure 5 Relationship between drug concentration in the effect compartment and median EEG frequency during the first infusion cycle in elderly subject No. 101.

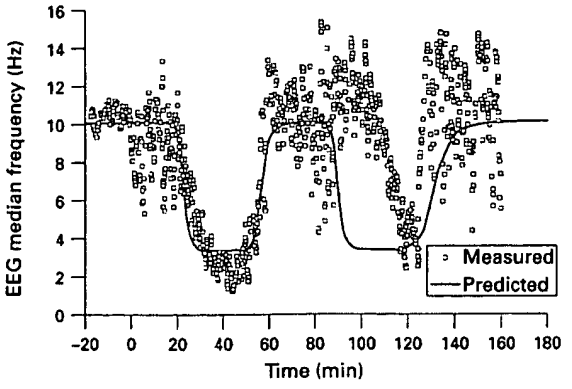

Figure 6 Median EEG frequency as a function of time during and after both infusion cycles in a young subject showing the poor fit of the data during the second infusion cycle.

appeared that during the second infusion cycle similar maximum drug effects occurred. Table 3 presents the pharmacodynamic variables estimated by fitting a sigmoid Emax function to the concentration–effect data of the first infusion cycle. Baseline values were slightly lower in the elderly than in the young subjects. Based on its EC_{50} values, Ro 48-6791 appeared to be a very potent benzodiazepine. Elderly subjects were more sensitive to the drug than young subjects. The concentration–effect relationship appeared to be steep, particularly in elderly subjects. Hysteresis between arterial plasma concentration and response was found in seven young and eight elderly subjects. Median k_{eq} values were similar in both groups. The equilibration half-life ($T_{1/2eq}$) was approximately 5–7 min. A representative plasma concentration–response plot during the first infusion cycle is given in figure 4 showing the hysteresis loop. Collapse of the hysteresis loop by incorporation of an effect compartment into the pharmacokinetic–pharmacodynamic model yields the effect compartment concentration–response plot of figure 5. In most subjects, the pharmacodynamic variable estimates of the first infusion cycle when applied to the second cycle led to less optimal characterization of the time course of effect in the latter (fig. 6).

Discussion

This is the second study in humans of the new benzodiazepine Ro 48-6791 which was developed to induce conscious sedation and anaesthesia, with or without other agents.[3]

Ro 48-6791 showed a favourable tolerability profile in both young and elderly subjects. There were only minor changes in cardiovascular and respiratory variables. Previous studies have shown that in this respect differences between various benzodiazepines are minor, even between long- and short-acting compounds.[16] It should be realized that in our study, high concentrations of Ro 48-6791 were attained at the pharmacodynamic end-point comparable with approximately 1200 μg litre^{-1} of midazolam. These concentrations are anticipated to be higher than those expected during therapeutic administration of Ro 48-6791. Anaesthetic conditions are seldom created by administration of a benzodiazepine alone. Monotreatment with Ro 48-6791 was used in this study to obtain information on tolerance, pharmacokinetics and pharmacodynamics. Obviously, Ro 48-6791 should still be studied in patients with compromised ventilatory function but this study did not provide indications that its profile would differ from that of midazolam.

The pharmacokinetics of Ro 48-6791 were described most accurately by an open three-compartment model. In this study the pharmacokinetics were examined by high-resolution techniques, involving frequent arterial blood sampling. This enabled accurate delineation of the distribution and elimination phases. In both groups clearance was markedly higher than that of midazolam.[17] In elderly subjects clearance was only approximately 15% lower than in young subjects, whereas the distribution rate constant from the

central to the shallow peripheral compartment (k_{12}) decreased by one-third. Little change in pharmacokinetics with age has also been reported with midazolam.[18,19] Both Ro 48-6791 and midazolam are metabolized oxidatively by isoenzymes of cytochrome P450. However, clearance of Ro 48-6791 is high and, therefore, more dependent on liver blood flow than on the oxidative capacity of the liver. The ratio of volume of distribution *vs* compartmental clearance with respect to the deep peripheral compartment (V_3/Cl_3) correlates inversely with the context-sensitive half-time after drug infusions that have not yet reached steady-state conditions.[20] The context-sensitive half-time is the time needed for a 50% reduction in drug concentration at the active site. This is because drug is still being taken up into the deep compartment thereby contributing to a reduction in concentration in plasma and the effect compartment. In contrast, a small Cl_3/Cl_1 ratio correlates with a shorter context-sensitive half-time after an infusion to steady state. This particularly reflects how rapidly drug leaves the deep compartment. With Ro 48-6791, V_3/Cl_3 and Cl_3/Cl_1 ratios were, on average, 17% larger and 40% smaller, respectively, than those for midazolam. A 30% faster recovery is usually considered as a change that is clinically discernible given the underlying variation in both pharmacokinetics and pharmacodynamics in a given patient.[20] These ratios indicate that, based on the pharmacokinetics of Ro 48-6791 *per se*, this drug might demonstrate a shorter duration of action than midazolam. The concentration–time courses in all subjects showed linearly ascending plasma concentrations during both infusion cycles providing evidence that the computer which controlled the infusion pump was programmed correctly, be it with two-compartment pharmacokinetic data. This study confirmed that Ro 48-6792 had a markedly longer elimination half-life than the parent compound. The sampling schedule did not allow accurate estimation of its half-life.

The pharmacodynamics of Ro 48-6791 were investigated using median EEG frequency as the effect measure. In contrast with drugs such as barbiturates and etomidate, infusion of benzodiazepines does not induce burst suppression. Therefore, a different pharmacodynamic end-point was chosen in this study. The EEG variable total number of waves between 12 and 30 Hz derived by aperiodic analysis has been used for pharmacokinetic–pharmacodynamic modelling studies with midazolam.[21] Bührer and colleagues derived the variable microvolts per second also from aperiodic analysis in the 0.5–30 Hz band.[22] Other authors have used the change in power determined by fast Fourier transform in the 12–30 Hz frequency band to investigate the pharmacodynamics of benzodiazepines.[23] Alternatively, the percentage change in alpha band activity (7.5–13.4 Hz) has also been used.[24] The difficulties inherent in the selection of a single univariate EEG variable to quantify the effects of benzodiazepines were illustrated by Fiset and colleagues[25] who used both aperiodic analysis and fast Fourier transform to describe the EEG effects of midazolam in a study in 10 subjects. In our study median EEG frequency was used as the effect measure as it is more reflective of the total effects of Ro 48-6791 on the EEG. Moreover, this variable has been used successfully in pharmacokinetic–pharmacodynamic modelling investigations with thiopentone, etomidate, propofol and eltanolone[26–28] and, therefore, has found wider use than variables based on aperiodic analysis. Median EEG frequency fulfilled virtually all prerequisites for an effect measure in kinetic–dynamic investigations.[29] Its clinical relevance needs to be demonstrated in studies which integrate several clinical end-points.

This study revealed a high potency for Ro 48-6791. The variability in EC_{50} was pronounced. This phenomenon has been described repeatedly for the pharmacodynamics of benzodiazepines.[6] Coefficients of variation of up to 50% have been reported by other research groups.[21,25,30] EC_{50} values in the range 180–384 μg litre^{-1} have been estimated for i.v. midazolam.[21,22,25] Mandema and colleagues reported a mean EC_{50} of only 77 μg litre^{-1}.[30] This illustrates on the one hand the marked variability in the pharmacodynamics of benzodiazepines or applied methodology, or both, and on the other hand the need to compare drugs or subject groups, or both, with the same strictly standardized method.[31] However, we can conclude that Ro 48-6791 is more potent than midazolam, in line with the findings of the first study in humans.[7] The potency of Ro 48-6791 was approximately 40% higher in elderly compared with young subjects. Increasing sensitivity of the central nervous system with age is often reported.[32] However, controlled clinical studies which take into account both pharmacokinetics and pharmacodynamics as sources of variations in drug response are rare. Greenblatt and colleagues described a linear concentration–effect relationship for triazolam in young and elderly individuals with no difference in intrinsic sensitivity between the two groups.[33] Another study reported increasing pharmacodynamic sensitivity to the hypnotic effects of midazolam in elderly subjects.[34] It is interesting to note that the influence of age on sensitivity appears to be more pronounced with inhalation anaesthetics than benzodiazepines.[35]

The difference in EC_{50} between young and elderly subjects in combination with the reduced clearance in elderly explain to a large extent the difference in total dose given to both groups to elicit the same pharmacodynamic end-point. Hysteresis in the plasma concentration–effect relationship was detected in most subjects. Median k_{eq} values in both groups were similar (8 and 6 h^{-1}). This implies a half-time of equilibration between plasma drug concentration and effect ($T_{1/2eq}$) of approximately 6 min. Values of 2–3 min have been reported in studies on midazolam with arterial blood sampling.[21,22] However, the EEG methodology in our study was different and, therefore, direct comparison between the two drugs is necessary. The established concentration–response relationships were steep, as reflected in the variable γ. In particular, the values obtained in elderly subjects were high. It remains to be investigated if midazolam shows the same phenomenon.

The pharmacodynamic variables estimated on the basis of the concentration–response data of the first infusion cycle poorly predicted the time course of effect during the second cycle. The second infusion appeared to induce the same decrease in median frequency but with a delayed onset. No major change was found in offset. There may be several explanations for this phenomenon. First, the influence of the metabolite Ro 48-6792 cannot be excluded. The more rapid cessation of drug effect would then suggest inverse agonistic activity of Ro 48-6792. However, various animal experiments investigating mixtures of Ro 48-6791 and Ro 48-6792 in different dose ratios did not provide support for this hypothesis (Jenck, personal communication, 1995). The first clinical study did not suggest pharmacodynamic effects of Ro 48-6792 but maximum plasma concentrations reached were only approximately 25 $\mu g\ litre^{-1}$. Alternatively, acute tolerance may develop to the high-dose EEG effects of Ro 48-6791 in the course of this study. The development of tolerance to benzodiazepines has been studied by Breimer[36] and Kroboth and Smith[37] who concluded that acute tolerance may occur. Other investigators did not obtain data indicative of acute tolerance development.[25,38]

Acknowledgements

We acknowledge the assistance of Dennis Thomsen and Manuela Wäfler in preparing this manuscript.

References

1. Hollister LE, Shader RI. Clinical uses of benzodiazepines. *Journal of Clinical Psychopharmacology* 1993; **13** (Suppl.): 1S–169S.
2. Bell GD. Review article: premedication and intravenous sedation for upper gastrointestinal endoscopy. *Alimentary Pharmacology and Therapeutics* 1990; **4**: 103–122.
3. Amrein R, Hetzel W, Allen SR. Co-induction of anaesthesia: the rationale. *European Journal of Anaesthesiology* 1995; **12** (Suppl. 12): 5–11.
4. Shelly MP, Sultan MA, Bodenham A, Park GR. Midazolam infusions in critically ill patients. *European Journal of Anaesthesiology* 1991; **8**: 21–27.
5. Dundee JW, Halliday NJ, Harper KW, Brogden RN. Midazolam: a review of its pharmacological properties and therapeutic use. *Drugs* 1984; **28**: 519–543.
6. Laurijssens BE, Greenblatt DJ. Pharmacokinetic–pharmacodynamic relationships for benzodiazepines. *Clinical Pharmacokinetics* 1996; **30**: 52–76.
7. Dingemanse J, van Gerven JMA, Schoemaker RC, Roncari G, Oberyé JJL, van Oostenbruggen MF, Massarella J, Segala P, Zell M, Cohen AF. Integrated pharmacokinetics and pharmacodynamics of Ro 48-6791, a new benzodiazepine, in comparison to midazolam during first administration to healthy male subjects. *British Journal of Clinical Pharmacology* 1997, in press.
8. Dingemanse J, Danhof M, Breimer DD. Pharmacokinetic–pharmacodynamic modelling of CNS drug effects: an overview. *Pharmacology and Therapeutics* 1988; **38**: 1–52.
9. Schüttler J, Schwilden H, Stoeckel H. Infusion strategies to investigate the pharmacokinetics and pharmacodynamics of hypnotic drugs: etomidate as an example. *European Journal of Anaesthesiology* 1985; **2**: 133–142.
10. Mastey V, Donati F, Varin F. Early pharmacokinetics of midazolam. Sampling site and schedule considerations. *Clinical Drug Investigation* 1995; **9**: 131–140.
11. Bell GD, Spickett GP, Reeve PA, Morden A, Logan RFA. Intravenous midazolam for upper gastrointestinal endoscopy: a study of 800 consecutive cases relating dose to age and sex of patient. *British Journal of Clinical Pharmacology* 1987; **23**: 241–244.
12. Scholer SG, Schafer DF, Potter JF. The effect of age on the relative potency of midazolam and diazepam for sedation in upper gastrointestinal endoscopy. *Journal of Clinical Gastroenterology* 1990; **12**: 145–147.
13. Gibaldi M, Perrier D. *Pharmacokinetics*, 2nd rev. Edn. New York: Marcel Dekker Inc, 1982.
14. Beal SL, Sheiner LB. *NONMEM User's Guide*, version IV. San Francisco: University of California, 1994.
15. Holford NHG, Sheiner LB. Kinetics of pharmacological response. *Pharmacology and Therapeutics* 1982; **16**: 143–166.
16. Ariano RE, Kassum DA, Aronson KJ. Comparison of sedative recovery time after midazolam versus diazepam administration. *Critical Care Medicine* 1994; **22**: 1492–1496.
17. Allonen H, Ziegler G, Klotz U. Midazolam kinetics. *Clinical Pharmacology and Therapeutics* 1981; **30**: 653–661.
18. Avram MJ, Fragen RJ, Caldwell NJ. Midazolam kinetics in women of two age groups. *Clinical Pharmacology and Therapeutics* 1983; **34**: 505–508.
19. Greenblatt DJ, Abernethy DR, Locniskar A, Harmatz JS, Limjuco RA, Shader RI. Effect of age, gender, and obesity on midazolam kinetics. *Anesthesiology* 1984; **61**: 27–35.
20. Youngs EJ, Shafer SL. Pharmacokinetic parameters relevant to recovery from opioids. *Anesthesiology* 1994; **81**: 833–842.
21. Breimer LTM, Hennis PJ, Burm AGL, Danhof M, Bovill JG, Spierdijk J, Vletter AA. Quantification of the EEG effect of midazolam by aperiodic analysis in volunteers: pharmacokinetic/pharmacodynamic modelling. *Clinical Pharmacokinetics* 1990; **18**: 245–253.
22. Bührer M, Maitre PO, Crevoisier C, Stanski DR. Electroencephalographic effects of benzodiazepines. II: pharmacodynamic modeling of the electroencephalographic effects of midazolam and diazepam. *Clinical Pharmacology and Therapeutics* 1990; **48**: 555–567.
23. Greenblatt DJ, Ehrenberg BL, Gunderman J, Locniskar A, Scavone JM, Harmatz JS, Shoder RI. Pharmacokinetic and electroencephalographic study of intravenous diazepam, midazolam, and placebo. *Clinical Pharmacology and Therapeutics* 1989; **45**: 356–365.
24. Koopmans R, Dingemanse J, Danhof M, Horsten GP, van Boxtel CJ. Pharmacokinetic–pharmacodynamic modeling of midazolam effects on the human central nervous system. *Clinical Pharmacology and Therapeutics* 1988; **44**: 14–22.
25. Fiset P, Lemmens HLM, Egan TE, Shafer SL, Stanski DR. Pharmacodynamic modeling of the electroencephalographic effects of flumazenil in healthy volunteers sedated with midazolam. *Clinical Pharmacology and Therapeutics* 1995; **58**: 567–582.
26. Hudson RJ, Stanski DR, Saidman LJ, Meathe E. A model for studying depth of anesthesia and acute tolerance of thiopental. *Anesthesiology* 1983; **59**: 301–308.
27. Schwilden H, Schüttler J, Stoeckel H. Quantitation of the EEG and pharmacodynamic modelling of hypnotic drugs: etomidate as an example. *European Journal of Anaesthesiology* 1985; **2**: 121–131.
28. Hering WJ, Ihmsen H, Langer H, Uhrlau C, Dinkel M, Geisslinger G, Schüttler J. Pharmacokinetic–pharmacodynamic modeling of the new steroid hypnotic eltanolone in healthy volunteers. *Anesthesiology* 1996; **85**: 1290–1299.
29. Dingemanse J. Kinetics and dynamics of drug effects on the nervous system. In: van Boxtel CJ, Holford NHG, Danhof M, eds. *The In Vivo Study of Drug Action. Principles and Applications of Kinetic–Dynamic Modelling*. Amsterdam: Elsevier, 1992; 113–132.
30. Mandema JW, Tuk B, van Steveninck AL, Breimer DD, Cohen AF, Danhof M. Pharmacokinetic–pharmacodynamic modeling of the central nervous system effects of midazolam and its main metabolite α-hydroxymidazolam in healthy volunteers. *Clinical Pharmacology and Therapeutics* 1992; **51**: 715–728.
31. Mandema JW, Danhof M. Electroencephalogram effect measures and relationships between pharmacokinetics and pharmacodynamics of centrally acting drugs. *Clinical Pharmacokinetics* 1992; **23**: 191–215.
32. Stijnen AM. The influence of ageing on the pharmacodynamics of sedative and anticonvulsant drugs in rats, PhD thesis. Leiden: University of Leiden, 1991.

33. Greenblatt DJ, Harmatz JS, Shapiro L, Engelhardt N, Gouthro TA, Shader RI. Sensitivity to triazolam in the elderly. *New England Journal of Medicine* 1991; **324**: 1691–1698.
34. Jacobs JR, Reves JG, Marty J, White WD, Bai SA, Smith LR. Aging increases pharmacodynamic sensitivity to the hypnotic effects of midazolam. *Anesthesia and Analgesia* 1995; **80**: 143–148.
35. Mapleson WW. Effect of age on MAC in humans: a meta-analysis. *British Journal of Anaesthesia* 1996; **76**: 179–185.
36. Breimer LTM. EEG effect during pseudo 'steady state' infusion of midazolam in volunteers: development of acute tolerance? In: Breimer LTM. *Interaction Between Midazolam and Flumazenil in Man: Pharmacokinetic-pharmacodynamic Modelling of the CNS Effect Using the EEG*. PhD thesis. Leiden: University of Leiden, 1991; 127–139.
37. Kroboth PD, Smith RB. Tolerance to alprazolam after intravenous bolus and continuous infusion: psychomotor and EEG effects. *Clinical Pharmacology and Therapeutics* 1988; **43**: 270–277.
38. Lauven PM, Schwilden H, Stoeckel H, Greenblatt DJ. The effects of a benzodiazepine antagonist Ro 15-1788 in the presence of stable concentrations of midazolam. *Anesthesiology* 1985; **63**: 61–64.